The Effects of Oxygen Tension and Glucose Concentration on the Metabolism of Porcine TMJ Disc Cells

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INTRODUCTION
The temporomandibular joint (TMJ) is a load-bearing joint consisting of the condyle of the mandibular bone, the fossa eminence of the temporal bone, and a fibrocartilaginous disc wedged in between. TMJ disc derangement and degeneration are the primary causes of TMJ disorders which have only recently been the focus of greater research. The TMJ disc is a large avascular structure, so required nutrients such as oxygen and glucose are supplied by blood vessels and synovial fluid at the margins of the disc. The nutrient transport within the TMJ disc mainly depends on diffusion and convection mechanisms. It has been postulated that sustained mechanical loading will decrease the nutritional levels in the disc. Studies have shown that articular cartilage is a physiologically hypoxic tissue with an oxygen gradient ranging from about 6% at the cartilage surface to <1% in the deepest layers. TMJ disc tissue has shown a similar oxygen gradient during physiological conditions, and has the potential for reaching even lower levels of oxygen tension under pathological loading conditions. Oxygen and glucose play critical roles in the metabolism of TMJ disc cells and are essential for both ATP production and matrix synthesis. Therefore, the objective of this study was to investigate the effect of glucose concentration on the energy metabolism and functions of porcine TMJ disc cells under different oxygen tension.

MATERIAL AND METHODS
Porcine TMJ discs were harvested under sterile conditions within 12 hours of death. The entire TMJ with capsule intact was removed en bloc, and overnight digested at 37 °C with 0.1% collagenase II. The first-passage (P1) cells were detached with trypsin-EDTA. The second (P2) cells were used for experiments and the viability of cells was quantified by trypan blue exclusion. At 90% confluence, the culture medium was replaced by the DMEM plus 10% FBS at 4 glucose concentrations (21%, 10%, 5%, and 1% O2). Cells were further cultured under various oxygen concentrations (21%, 10%, 5%, and 1% O2) for 48 hours in a Galaxy triple gas incubator in which N2 was used as a replacement for O2 levels.

Cell viability and proliferation of experimental groups was measured using WST-1 kit (Roche Molecular Biochemicals, Mannheim, Germany). Levels of ATP in the cells were determined according to the luciferin-luciferase (PerkinElmer, Wellesley, MA). Lactate Assay kits were purchased from Eton Bioscience Inc. Cellular synthesis of collagen and proteoglycans was determined by measuring the incorporation of radioactivity (derived from [2,3-3H] proline and 35S-labeled SO4) into collagen and glycosaminoglycans (GAG).

The results from three independent trials were statistically analyzed. Comparisons were performed by Two-way analysis of variance with Tukey’s LSD as a post hoc test. P-values of less than 0.05 were considered to be significant.

RESULTS
The WST assays show that the proliferation rate of TMJ disc cells decreased as glucose concentrations decreased, while it generally increased as oxygen concentration decreased (Fig 1.1). The release of lactate was increased with the increase of glucose concentration. The production rates of lactate were also oxygen dependent with maximum rate at 5% oxygen (Fig 1.2). The ATP production generally decreased as oxygen tension decreased. At 0.25g/L glucose, the TMJ disc cells reached the highest ATP production rate for all tested oxygen tensions (Fig 1.3). The collagen synthesis rate increased as glucose concentration increased, but decreased as oxygen concentration decreased (Fig 2.1). The GAG synthesis rate was increased with glucose concentration increases, and decreased with oxygen tension decreases (Fig 2.2).

DISCUSSION
The effects of oxygen tension and glucose concentration on the metabolism of porcine TMJ disc cells were investigated. Our results show that TMJ disc cell proliferation was enhanced at lower oxygen tension, which is similar to the cartilage chondrocytes. However, unlike cartilage chondrocytes, TMJ disc cells appear to show a so-called negative Pasteur Effect in which glycolysis falls as oxygen levels drop, contributing to the fall in ATP. Consequently, the matrix synthesis rates of TMJ disc cells decreased as oxygen tension decreased, since the matrix synthesis is closely coupled to intracellular ATP levels. The possible reason is that TMJ disc has multiple cell types with about 70% fibroblastic cells.

Nutrient supply is thought to be a factor limiting cell density in the avascular tissues, such as TMJ disc. The maintenance of oxygen and glucose homeostasis is essential for many vital cellular functions including division, proliferation, differentiation, excitability, and secretion. The sustained mechanical loading on TMJ disc will induce both oxygen and glucose concentrations decrease towards the center of the TMJ disc. The results of this study therefore support the idea that a fall in nutrient supply might be one pathway to disc degeneration due to mechanical loading.

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Figure 1. (1) WST-1 results of TMJ disc cells at various glucose and oxygen concentrations; (2) Lactate production; (3) ATP production.

Figure 2. (1) Collagen synthesis rate of disc cells at various glucose and oxygen concentrations; (2) GAG synthesis rate.